Fatty Acids of Lard. B. Quantitative Estimation by Silicic Acid and Gas-Liquid Chromatography[1]

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Abstract

A quantitative comparison was made of the fatty acid composition of a commercially rendered lard, a laboratory rendered lard, and a solvent extracted lard representing the same batch of pig tissue. The composition was determined by a preliminary separation of the methyl esters on a silicic acid column followed by gas-liquid chromatography of the fractions containing a known added amount of reference compound. This technique permitted estimation of the composition of each fraction and subsequent calculation of percentage of the minor as well as the major components in the original lard. It was concluded that the minor components were not artifacts produced in rendering.

Introduction

IMPROVED TECHNIQUES in application of gas-liquid chromatography (GLC) for separating fatty acid esters have shown that natural fats are more complex than was previously reported. Numerous minor components are being found which had previously escaped detection. However, some natural fats (1,2,4, 6,7,9,10) contain such a multiplicity of minor components, many of which have essentially the same retention on a given polyester column, that even the use of several columns with different stationary phases will fail to resolve and permit detection of all the components. Hence, some auxiliary means of fractionation followed by gas-liquid chromatography on each of these fractions is sometimes necessary. Furthermore, some fatty acids are present in such low concentrations that preliminary fractionation is required to effect sufficient concentration for detection by GLC.

In recent work from this laboratory on fatty acids of lard (Part A) (3) attention was given primarily to identification of minor fatty acids in lard. A nonpolar silicone column was employed to separate the fatty acid methyl esters mainly on the basis of chain length. Fractions were collected and rechromatographed on a polyester (EGS) column. This technique permitted detection of many minor or trace components which had not been reported in previous work. No attempt, however, was made to estimate percentage distribution owing to uncertainties concerning quantitation in collection of fractions.

In the present work attention was given to quantitative aspects. Preliminary fractionation of the lard methyl esters was made by silicic acid column adsorption chromatography. The fractions thus obtained, after the addition of a known amount of reference ester to each, were then subjected to GLC on a polyester column. The percentage fatty acid distribution in the total lard, determined by summation of data from the fractions, is presented for three lards obtained from the same batch of tissues. The same fatty acids were found in each specimen in about the same order of percentage. It was concluded that the minor components were not artifacts produced in rendering.

Experimental

Samples. A fresh sample of steam-rendered lard was obtained from a local packer on the same day it was rendered. Fresh pig tissues representative of the commercial batch being rendered were also obtained and were frozen until they could be processed in the laboratory. The tissues were minced, mixed, and divided into two parts. One part was carefully rendered in a glass vessel under nitrogen at 115–120C; the other was thoroughly extracted with redistilled acetone, methylal-methanol (4:1), and hexane at room temperature. The solvents were removed from the filtered extracts in a rotary evaporator under reduced pressure.

The commercially rendered lard and the two corresponding lard samples produced in the laboratory were converted to methyl esters, and freed of unsaponifiables by silicic acid column treatment (8).

GLC Apparatus. The apparatus and thermal conductivity cell detector employed were the same as described previously (5).

GLC Columns. The separations were performed at a temperature of 212C with a stainless steel coiled column 8 ft × 3/16 in. OD (0.118 in. ID) and packed with acid and base washed Chromosorb "W" (42–60 mesh) coated with 25% ethylene glycol succinate polyester. The treatment of the support and preparation of the polyester has been described (4).

Adsorption Columns. A slurry of 3.5 g of 80% silicic acid-20% filter aid in hexane was poured into a conventional chromatographic column 12 mm × 250 mm. When the packing was properly settled, 30–40 mg of lard methyl esters were introduced onto the column and eluted. Three fractions were obtained by elution with 50 ml each of hexane and a final fraction eluted with 100 ml of an equal mixture of hexane and ethyl ether.

Quantitation. A known amount of highly purified 15:0[3] methyl ester was added to each fraction from the adsorption column separation and then each was subjected to GLC analysis. The area under the peak on the chromatogram of the individual components in each fraction was measured and made relative to that of the reference ester. Since the area representing the reference ester is equivalent to a known weight, the weight of each component could be calculated. When these weights from all of the fractions were summated the result was the total weight of lard methyl esters and from these data percentages were calculated. The composition of the lard methyl esters was also determined directly from the areas of the peaks on the chromatogram of the total esters without the use of an internal standard.

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[3] A "shorthand" designation of structure of each fatty acid is used throughout this paper. 18:0 = saturated C_{18} acid; 18:2 = C_{18} with 2 double bonds; 18:0 br = branched chain C_{18} acid, etc.

Results and Discussion

In an earlier publication (5), it was not possible to determine tetraenes and pentaenes by GLC on the total esters of lard with the column and conditions employed; however, with the present narrower diameter column and operating conditions it was possible to estimate the amount of 20:4 (arachidonic acid) but not the 20:5 nor the unsaturated C_{22} acids. Chromatograms of the total methyl esters of the three lards were essentially identical and are represented by Figure 1. The chromatogram was obtained by injecting a relatively large sample, actually a slight overload, in order to show the presence of many of the minor components. Notice that the peak due to the presence of a mixture of 14:1 and 15:0 (peak 4) can readily be seen although together they amount to only about 0.03%. By way of contrast, about seven times as much of 20:4 (peak 15) is difficult to detect since its peak, occurring later on the chromatogram, is broad but has very little height owing to its dilution with carrier gas as it slowly moves off the column. For the same reason, none of the C_{22} esters are seen. The C_{19} esters are completely coincident with the peaks of the C_{18} unsaturated esters.

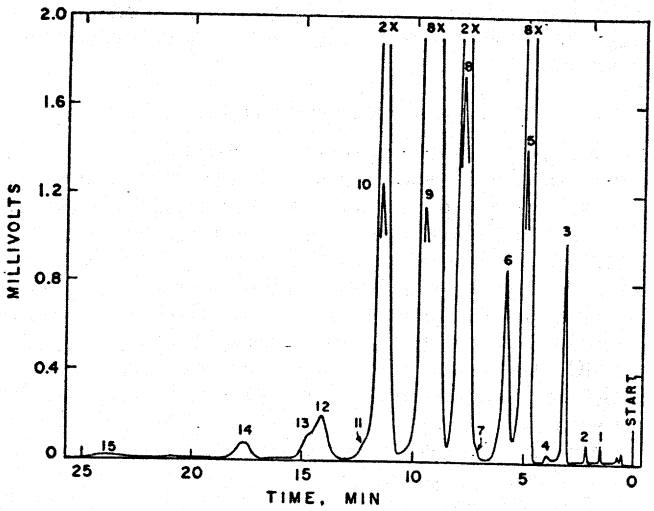

FIG. 1. Chromatogram of the total esters of lard. Column 8 ft × 3/16 in., temp 212C, 25% EGS polyester. Peak identification: 1. 10:0; 2. 12:0; 3. 14:0; 4. 14:1 + 15:0; 5. 16:0; 6. 16:1 + 17:0; 7. 17:1; 8. 18:0; 9. 18:1; 10. 18:2; 11. 20:0; 12, 20:1; 13. 18:3; 14. 20:2; 15. 20:4. 2X and 8X = attenuation.

In order to separate esters with similar retentions by GLC and to concentrate the minor components, it was necessary to employ auxiliary fractionation. In previous work on milk fatty acids (4), silicic acid adsorption chromatography was shown to be effective for this purpose. It was employed successfully in the present investigation. Four fractions were obtained, the first, amounting to approximately 1/3 of the 30–40 mg sample applied to the column, consisted mainly of saturated esters. Qualitative evidence was also found for traces of hydrocarbons in this fraction. The second fraction, about 1/2 the sample, consisted principally of saturated and monounsaturated esters. The balance of the sample was about equally distributed in a third and fourth fraction; the third contained the monounsaturated plus diunsaturated esters and the fourth the diunsaturated plus the more highly unsaturated esters.

It was not necessary to weigh each fraction from the silicic acid separation since the addition of a known quantity of a reference ester as an internal standard to each fraction permitted calculation of the amount of each component from the gas-liquid chromatogram. The center of a chromatogram is the optimum position for a peak representing the internal standard. Owing to the multiplicity of components in lard, this position was not suitable and the 15:0 methyl ester, even though appearing early on the chromatogram, was chosen because it offered the least interference in the calculation of the fatty acid components. Although 15:0 is present in lard, as shown on the chromatogram of the total esters (Fig. 1, peak 4) it occurs only to the extent of 0.02 or 0.03% and therefore does not materially affect the results. We added the internal standard to each fraction in an amount equal to 5–10%.

The peaks on the chromatograms were identified by comparison with reference esters and from a plot of carbon chain lengths for a homologous series of compounds against the logarithm of their relative retention times. Each fraction was chromatographed before and after hydrogenation as a further check on components believed to be unsaturated esters. All acids reported in lard in the previous publication (3) were found in this study in an amount such that a quantitative figure could be assigned to them with the exception of 22:0, 22:1, 22:2, and 22:3. These acids are present in such small amounts that additional concentration of them would be necessary for a quantitative assignment.

The composition of the three lards determined from GLC analyses of the fractions obtained from the silicic acid column separation are shown in Table I along with GLC analyses based on the chromatograms of the total lard esters. For those components that appear on the chromatograms of the total lard esters, the percentage values show reasonable agreement with those obtained by summation of analyses of their fractions. Obviously more components were determined

TABLE I

Composition of Three Lards from GLC Analysis on Total Esters and from Fractions Obtained by Silicic Acid Separation

Component	Commercial lard		Lab. rendered lard		Solv. extracted lard	
	Total[a]	Fraction[b]	Total[a]	Fraction[b]	Total[a]	Fraction[b]
	%	%	%	%	%	%
10:0	0.04	0.01	0.05	0.02	0.04	0.01
12:0	0.07	0.04	0.06	0.05	0.06	0.05
14:0	1.17	1.09	1.17	1.11	1.24	1.06
14:1	0.06	0.02	0.03	.....	0.04	0.01
15:0		0.02		0.02		0.03
16:0	26.4	25.7	26.2	24.8	27.0	27.6
16:1	2.64	2.20	2.16	1.87	2.04	1.74
17:0		0.45		0.21		0.40
17:1	0.28	0.22	0.20	0.29	0.21	0.10
18:0 br	.....	trace	.....	trace	.....	trace
18:0	10.2	12.0	12.5	13.3	11.6	14.4
18:1	47.3	45.2	46.4	45.1	46.5	42.0
18:2	9.52	10.4	9.05	10.6	8.98	9.93
conj. 18:2 *c,t*[c]	.....	0.18	.....	0.09	.....	0.09
conj. 18:2 *t,t*[c]	.....	0.08	.....	0.04	.....	0.04
18:3	0.52	0.34	0.49	0.44	0.56	0.42
19:0	.....	0.04	.....	0.03	.....	0.04
19:1	.....	0.12	.....	0.07	.....	0.08
20:0	0.37	0.18	0.32	0.17	0.35	0.20
20:1	0.93	0.72	0.87	0.87	0.93	0.89
20:2	0.37	0.37	0.40	0.45	0.39	0.38
20:3	trace	0.11	trace	0.07	trace	0.09
20:4	0.16	0.20	0.20	0.20	0.15	0.21
20:5	.....	0.09	.....	0.05	.....	0.09
22:0	.....	trace	.....	.....	.....	0.01
22:2	.....	0.02	.....	.....	.....	.....
22:4	.....	0.05	.....	0.05	.....	0.05
22:5	.....	0.04	.....	0.03	.....	0.02
Unknown	.....	0.03	.....	0.02	.....	0.03

[a] Calculated from a chromatogram of the total lard methyl esters.
[b] Calculated from chromatograms of fractions obtained by silicic acid column separation of lard methyl esters and use of internal standard.
[c] *c,t* and *t,t* = *cis,trans* and *trans,trans*.

on these ester fractions than was possible on the total lard esters. The greatest numerical variations in percentages are those for methyl stearate and methyl oleate. This may be due mainly to normal experimental errors in measuring the peak areas, since one or the other of these esters is present in three fractions in amounts requiring attenuation of the detector signal. It should be noted that although one lard was commercially rendered, another rendered in the laboratory, and the third solvent extracted each contained the same components. It is concluded, therefore, that the components are naturally occurring and not produced as artifacts in the rendering process.

A comparison of the polyunsaturated acids found in the three lards as determined by GLC on the fractions and by the ultraviolet spectrophotometric method on the total esters is shown in Table II. Good agreement was obtained even for the tetraenes and pentaenes. The small differences in composition between the three lards by the fractionation procedure are not considered significant and probably are the result of normal errors involved in measuring peak areas and in adding the internal standard. The use of auxiliary fractionation by silicic acid and the use of GLC on the fractions obtained affords a means of quantitative estimation of minor components as well as major components of complex fats.

TABLE II

Polyunsaturated Acid Composition of Three Lards by GLC and U.V. Spectrophotometry

	Commercial lard		Lab. rendered lard		Solv. extracted lard	
	U.V.[a]	GLC[b]	U.V.[a]	GLC[b]	U.V.[a]	GLC[b]
	%	%	%	%	%	%
Diene	12.2	11.1	11.6	11.2	11.6	10.4
Triene	0.87	0.45	0.78	0.51	0.89	0.51
Tetraene	0.35	0.25	0.26	0.24	0.27	0.26
Pentaene	0.15	0.13	0.11	0.09	0.12	0.12

[a] Determined on total lard methyl esters.
[b] Calculated from chromatograms of fractions obtained by silicic acid column separation of lard methyl esters and use of internal standard.

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